

Immunotoxins for the Treatment of B-Cell Lymphomas

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INTRODUCTION

Non-Hodgkin's lymphoma (NHL) encompasses a group of hematologic malignancies of B and T cell origin. However, the majority of NHLs are B cell lymphomas and these can further be categorized as indolent or low grade, intermediate grade, and aggressive or high grade (1–3). There are approximately 30,000–50,000 new cases of B cell lymphoma in the United States each year; a large proportion of diagnosed patients will eventually die of this disease despite conventional chemo- and radiotherapy as well as bone marrow transplantation (in refractory or relapsed NHL).

Since NHLs typically express one or more B cell markers, these markers can be used to target antibody-based cytotoxic agents. Although normal B cells will be destroyed, they are repopulated from stem cells lacking the targeted antigens. Alternatively, since B cell tumors are clonal, the immunoglobulin idiotype can be considered a tumor-specific marker. In mice with human lymphoma xenografts, antibodies conjugated to radionuclides, drugs, or toxins can be curative, particularly when combined with other therapies. These immunoconjugates are highly potent *in vitro* and in mice. However, because they carry a toxic moiety, their safety profile in humans must be carefully established. In the case of antibody toxin conjugates (immunotoxins, or ITs), these agents have displayed better antilymphoma activity at lower concentrations than did unconjugated antibodies both *in vitro*

and *in vivo*. Early trials using ITs in patients have established the safe doses and the side effects, and efficacy must now be established in Phase II and III trials. Clearly, ITs have activity in humans but it remains to be determined whether they will improve the long-term prognosis for patients with NHL.

IMMUNOTOXINS

Two major antigens on B lymphoma cells have been used as targets; CD19 and CD22. Antibodies against these two molecules have been conjugated to either ricin toxin (RT) (4) or its deglycosylated A chain (dgRTA) (5–8), or pokeweed antiviral protein (PAP) (9).

dgRTA Containing ITs (Fig. 1)

CD19 is expressed on all normal B cells from the pre-B cell stage to the plasma cell (10–12), whereas CD22 appears on the mature B cell and disappears about the same time as CD19 on activated cells (10,11). CD19 and CD22 are expressed on greater than 90% and 60–80% of B lymphomas, respectively. Both anti-CD19 and anti-CD22 have been conjugated to dgRTA and in the case of RFB4-anti-CD22, the monoclonal antibody (MAb) has been used as both intact IgGs (13) and Fab' (14) fragments. dgRTA is produced by deglycosylating the whole molecule of RT followed by separation of the two chains by size and affinity chromatography (15,16). The removal of the carbohydrate moieties from the RTA avoids the problem of liver entrapment and hepatotoxicity. dgRTA as well as other ribosome inactivating proteins (RIPs) from plants, i.e., PAP, Saporin, etc., are glycosidases that inhibit

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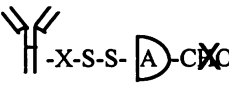
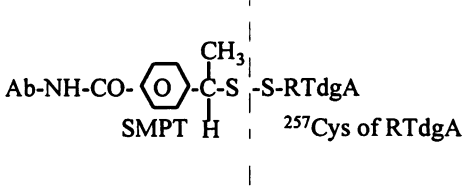
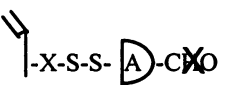
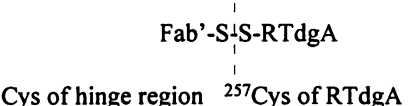
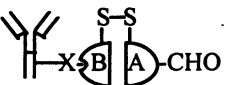
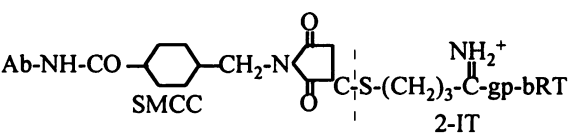
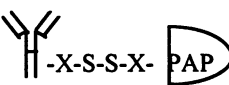
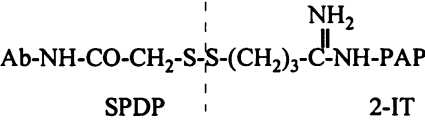
NAME	IT	CROSSLINKER
Antibody-RTdgA Ab: HD37, RFB4		
Fab'-RTdgA Ab: RFB4		
Antibody-bRT Ab: B43		
Antibody-PAP Ab: B4		

FIG. 1. Structure of immunotoxins used in the treatment of lymphomas

Antibodies HD37, B43, and B4 are murine monoclonals anti-CD19. Antibody RFB4 is a murine monoclonal anti-CD22. Abbreviations: A, ricin A chain; Ab, antibody; B, ricin B chain; CHO, carbohydrate moiety; gp, glycopeptide derived from fetuin; 2-IT, 2-imminothiolane; S-S, disulfide bond; X, cross-linking bridge.

protein synthesis in targeted cells by removing a specific adenine from the ribosomal RNA (17). The RFB4 anti-CD22 and HD37 anti-CD19 do not recognize cells other than B cells. The RFB4 and HD37 MAbs conjugated to dgRTA kill human neoplastic B cells that express the relevant markers. When Daudi cells are used as targets, RFB4-IgG-dgRTA has an IC_{50} of 10^{-12} M (13) and is therefore approximately 10-fold more potent than its Fab' fragment conjugated to the same toxin ($IC_{50} = 10^{-11}$ M) (14). In contrast, the HD37-IgG-RTA has an IC_{50} of $1-5 \times 10^{-11}$ M (13) and is 10- to 50-fold less cytotoxic than RFB4-IgG-dgRTA. Recombinant RTA (rRTA),

lacking all carbohydrates, may also be used for chemical construction of ITs (18–20).

Blocked Ricin (bRT)–Containing ITs

The anti-CD19 monoclonal antibody B4 has been conjugated to bRT. The bRT is prepared by chemically blocking the galactose-binding sites on the ricin toxin B chain (RTB) with ligands containing N-linked oligosaccharides derived from fetuin (5–8,21). The MAb is treated with the SMCC cross-linker to establish a thioether bond between the MAb and the galactose-containing oligosaccharide provided with a sulfhy-

dryl group by treatment with 2-iminothiolane (6,21). The bRT-containing IT molecule is unable to bind to cells other than target cells (22). The in vitro cytotoxicity of B4-bRT has been tested on CD19⁺ human Burkitt's lymphoma cell lines, where it was found to be 5-fold more potent than the HD37-dgRTA (4).

PAP-Containing ITs

The anti-CD19 murine monoclonal antibody B43 has been conjugated to PAP following derivatization with the *N*-succinimidyl-3-(2-pyridyldithiol)propionate (SPDP) cross-linker and 2-iminothiokene-treated PAP (23). Its in vitro cytotoxicity on Nalm-6 cells was relatively low with an IC₅₀ approaching 10⁻⁹ M (9,23). It is therefore 50- to 100-fold weaker than HD37-dgRTA and 1,000-fold weaker than the B4-BRT conjugate. Nevertheless, the B43 PAP has good activity in vivo (24).

TREATMENT OF MICE WITH HUMAN LYMPHOMAS (TABLE 1)

Animal Models

Human xenografts have been successfully grown in immunodeficient mice lacking T cells (Nude mice) or both T and B cells (SCID mice). Lymphoma cells are injected either subcutaneously, where they grow as solid tumors (25), or intravenously, where they grow in a disseminated fashion more akin to human lymphomas (26). Disseminated tumors are present in the lung, kidney, ovary, liver, spleen, and the vertebral column of the mice. The growth of disseminated tumor in the spinal canal causes paralysis of the animal shortly prior to death. Both survival and mean paralysis time (MPT), which is predictive of death, have been used as end points in this animal model (26,27).

Treatment of Xenografted Mice

Xenografted mice are treated in one of two ways. For minimal residual disease (MRD), the mice are inoculated with tumor cells approximately 24 hr prior to commencing therapy. For more advanced disease, mice that would normally survive for 30–50 days following inoculation with tumor cells are treated 7–21 days after tumor cell inoculation. In mice with solid subcutaneous tumors, ITs are administered when the tumor has a measurable diameter of 1 cm or less.

Therapeutic Effects of ITs

Single ITs or mixtures of ITs have also been administered to tumor-bearing mice alone or in conjunction with chemotherapy (20,25,28–36). ITs prepared with either anti-CD22 or anti-CD19 and any one of the three toxins mentioned above have significant antitumor activity. In general, a combination of the two ITs exerts the best effect (29). Although therapy with ITs alone can be highly effective, it is rarely curative in all animals, even when administered shortly after injection with tumor cells. However, when ITs are combined with chemotherapy in early disease, the effects are curative. In more advanced disease, ITs have significant antitumor activity but are not curative even when administered with chemotherapy. Interestingly, in advanced disease, ITs administered before or during chemotherapy are more therapeutic than when they are given after chemotherapy (31). This suggests that the IT may sensitize the tumor to chemotherapeutic agents. Taken together, the mouse studies have demonstrated that ITs will probably work best in the setting of MRD, possibly when administered before or in conjunction with chemotherapy.

THERAPY OF LYMPHOMA PATIENTS (TABLE 2)

Although studies in mice suggest that ITs should be administered as cocktails along with chemotherapy in the setting of MRD, it is not possible to do this in humans until Phase I safety criteria are established. Therefore, to date, virtually all patients treated with ITs have been end-stage multiply relapsed patients often with bulky disease. Because of this, it has not yet been possible to determine how effectively ITs will work in an optimal setting. However, a number of Phase I trials have clearly defined the side effects, safe dosages, and pharmacokinetics of these targeted therapies in humans and have indicated that the ITs do indeed have antitumor activity in humans (37–43).

dgRTA ITs

The three dgRTA-based ITs that have been used in Phase I trials are the Fab'-RFB4-dgRTA, the IgG-RFB4-dgRTA, and the HD37-dgRTA. The ITs have been administered either by continuous infusion (c.i.) over 8 days or 24 hr or by bolus infusion (b.i.) every other day for a total of four

TABLE 1. Effect of various ITS in mice with human lymphoma xenografts

Specificity	Tumor Cells	Mice	Tumor Type	IT	Treatment	Therapeutic Effect	Reference
Anti-CD22	CA46	Nude	Solid	RFB4-PE35	Early ^a	Prevents tumor growth	(25)
	Ramos	Nude	Solid	CLB-B-ly/1-rRTA	Late ^b	Temporary reduction of tumor growth	(20)
	Ramos	SCID	Disseminated	CLB-B-ly/1-rRTA	Late	Tumor-free animals at 175 days	(20)
	Daudi	SCID	Disseminated	RFB4-RTdgA	Early	MPT (31 → 67 days)	(28)
					Late	MPT (26 → 54 days)	(28)
	Daudi	SCID	Disseminated	RFB4-RTdgA + HD37-RTdgA	Early	MPT (30 → 103 days)	(29)
	Daudi	SCID	Disseminated	RFB4-RTdgA + HD37-RTdgA + Doxorubicin	Early	Tumor-free animals at 150 days	(30)
					Late	MPT (30 → 120 days)	(31)
Anti-CD19	Namalwa	SCID	Disseminated	B4-bRT	Early	ST50 from 9 → 46 days	(32)
	(i.p.)	SCID	Disseminated	B4	Early	ST50 from 9 → 36 days	(32)
	Nalm-6	SCID	Disseminated	B4-bRT	Late	ST50 from 25 → 30 days	(32)
	Namalwa	SCID	Disseminated	B4-bRt	Late	ST50 from 27 → 41 days	(32)
				B4	Late	No effect	(32)
	Namalwa	SCID	Disseminated	B4-bRT	Late	MST from 23 → 33 days	(33)
				B4-bRT + Doxorubicin	Late	MST from 23 → 48 days	(33)
	Daudi	SCID	Disseminated	HD37-RTdgA	Early	MPT from 30 → 55	(29)
				HD37-RTdgA + RFB4 [F(ab') ₂]	Early	Tumor-free animals at 1 year	(27)
	Nalm-6-UM1	SCID	Disseminated	B43-PAP	Early	38% mice, MST from 38 → 74 days	(34)
				(high dose)		62% mice, MST from 38 → 210 days	(34)
				B43-PAP + Cyclophosphamide	Early	90% mice, MST from 38 → 74 days	(35)
	Nalm-6	SCID	Disseminated	BU12-SAP	Late	40% tumor-free animals at 110 days	(36)

Abbreviations: rRTA, recombinant RTA; RTdgA, deglycosylated RTA; MPT: mean paralysis time; MST, mean survival time; ST50, survival time for 50% of animals treated or not treated with IT; PAP, pokeweed antiviral protein; SAP, Saporin; bRT, blocked ricin toxin.

^aEarly treatment: administration of IT 24 hr after inoculation of tumor cells.

^bLate treatment: administration of IT 7 or more days after inoculation of cells.

TABLE 2. Clinical trials with anti-CD19 or anti-CD22 ITs in patients with relapsed lymphoma

Toxin	Antibody ^a	Infusion	No. of Patients Evaluable	Dose-Limiting Toxicity	IT Half-Life (hr)	MTD	Clinical Response		Anti-Mouse IgG/Anti-Toxin Antibody Response (%)	Reference
							CR ^b	PR ^c		
dgRTA	RFB4 (Fab')	b.i.	14	VLS, ^d rhabdomyolysis	1.5	75 mg/m ²	0	5	36	(37)
	RFB4 (IgG)	b.i.	24	VLS, rhabdomyolysis	7.8	20 mg/m ²	1	5	62.5	(38)
	RFB4 (IgG)	c.i.	16	VLS	10.7	20 mg/m ²	4	0	75	(39)
	HD37	b.i.	23	VLS, rhabdomyolysis	18.2	16 mg/m ²	1	1	25	(40)
bRT	HD37	c.i.	9	VLS, acrocyanosis	22.8	19 mg/m ²	0	1	30	(40)
	B4	b.i.	7	VLS, hypotension	17.0	8 mg/m ²	0	0	33	(41)
	B4	b.i.	25	Liver dysfunction, thrombocytopenia	NR ^e	50 µg/Kg/d × 5	1	2	48	(42)
	B4	b.i.	34	As above plus myalgias	NR	50 µg/Kg/d × 7	2	3	53	(42)
PAP	B4	c.i.	12 ^f	Thrombocytopenia, arthralgias	NR	40 µg/Kg/d (2 cycles every 28 days)	8 ^g	0	58	(42)
	B4	c.i.	49 ^f	Anorexia, myalgias	NR	30 µg/Kg/d (2 cycles every 14 days)	30 ^g	0	49	(42)
PAP	B43	b.i.	30	VLS	14.0	100 µg/Kg/day × 5	NR	NR	NR	(43)

^aAntibodies HD37, B43, and B4 are murine MABs anti-CD19; RFB4 is a murine MAB anti-CD22.^bCR, complete response.^cPR, partial response.^dVLS, vascular leak syndrome.^eNR, not reported.^fTreatment given to patients following bone marrow transplantation.^gAfter bone marrow transplantation.

doses (37–41). In addition, a combination of the two ITs have been given by c.i. over 8 days. The following can be concluded from the Phase I trials: (1) The maximum tolerated dose (MTDs) ranges from 15 to 30 mg/m² (600–900 µg/kg), depending on the dose regimen. In general, the MTD is slightly lower for the c.i. regimen than for the b.i. regimen and higher for patients with circulating tumor cells using either regimen. (2) Side effects include manifestations of vascular leak syndrome (VLS) as well as myalgias. In most patients, these side effects are reversible following the completion of therapy. (3) About one-third of the patients make anti-IT antibodies after a single course of IT and up to 40–50% make antibodies after four or more courses. Antibody responses are often low and take 30–60 days to develop after therapy. Nevertheless, in patients who do not make antibodies, up to five or six courses can be given. (4) Clinical responses are observed in 15–40% of the patients and generally are best in those patients with the smallest tumor burdens. Given the results of the Phase I trials, the strategy for Phase II and III trials includes the administration of a mixture of the two ITs by b.i. at a dose of approximately 10–15 mg/m². If the response rate in the Phase II trials is good, Phase III trials will determine whether ITs can indeed be of benefit to patients with NHL.

bRT-based ITs

The B4-bRT IT has been evaluated in Phase I and II trials (42) and is now being studied in a large Phase III trial. When administered either daily by a 1-hr infusion for 5 consecutive days or continuously over 7 days to patients with B lymphoma, the MTDs were 250 and 350 µg/Kg, respectively. The side effects consisted of transient hepatotoxicity and thrombocytopenia. The complete responses (CRs) were of brief duration because of the small doses delivered to patients with large tumor burdens. To use the IT in a more desirable setting, it has now been administered as adjuvant therapy in patients with relapsed NHL following autologous bone marrow transplantation. No new toxicities were observed with one or more courses of therapy and only 2 of 38 patients developed thrombocytopenia. Thirty patients remain in CR for a median follow-up of 15 months.

A Phase III clinical trial is ongoing in patients who achieve CR after bone marrow transplantation. To date, the patients have received two courses of B4-bRT IT or no further therapy.

PAP-based ITs

The B43-PAP IT has been evaluated in a Phase I dose-escalation trial to 30 patients with leukemia (43). The dose level ranged from 0.1 µg to 250 µg/Kg/day for 5 consecutive days and 100 µg/Kg/day × 5 has been identified as the safe dosage level for further studies. A total of 16 patients were treated with this dosage. Vascular leak with high serum creatine and bilirubin was observed. Clinical responses were not reported but it was found that the anti-leukemic response was greater in patients with greater systemic exposure to IT and a lower peripheral blast count.

FUTURE PERSPECTIVES

The development of ITs is a lengthy and complex process and the optimization of ITs for cancer therapy is even more complex, but there is every reason to believe that success for at least some tumors will be achieved in the next 5 to 10 years. In fact, for the therapy of cancer, ITs have yielded higher response rates in Phase I/II trials than have some of the drugs used today (when tested in similar trials). The generation of new constructs, combinatorial therapy, and, in the case of cancer therapy, treatment of tumors that are amenable to IT-mediated killing (e.g., MRD) should eventually result in effective treatment protocols.

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